

AMENDMENTS TO THE CLAIMS:

This listing of the claims will replace all prior versions and listings of claims in the application.

Listing of Claims:

1-25. (canceled)

26. (previously presented) A method for detecting the presence of a target nucleic acid molecule in a sample, comprising:

- a) incubating a sample with a thermostable 5' nuclease under conditions wherein a cleavage structure is formed, said cleavage structure comprising:
 - i) a synthetic target nucleic acid, said synthetic target nucleic acid comprising a first region and a second region, said second region downstream of and contiguous to said first region;
 - ii) a first nucleic acid molecule, wherein at least a portion of said first nucleic acid molecule is completely complementary to said first region of said target nucleic acid;
 - iii) a second nucleic acid molecule comprising a 3' portion and a 5' portion, wherein said 5' portion is completely complementary to said second region of said target nucleic acid;

wherein said thermostable 5' nuclease lacks synthesis activity, and wherein at least a portion of said first nucleic acid molecule is annealed to said first region of said synthetic target nucleic acid, and wherein at least a portion of said second nucleic acid molecule is annealed to said second region of said synthetic target nucleic acid;

- b) cleaving said cleavage structure with said thermostable 5' nuclease so as to generate non-target cleavage product; and
- c) detecting the cleavage of said cleavage structure.

27. (previously presented) The method of Claim 26, wherein said detecting the cleavage of said cleavage structure comprises detecting said non-target cleavage product.

28. (previously presented) The method of Claim 26, wherein said synthetic target nucleic acid comprises an amplified nucleic acid.

29. (previously presented) The method of Claim 28, wherein said amplified nucleic acid is produced using a polymerase chain reaction.

30. (previously presented) The method of Claim 26, wherein said detecting the cleavage of said cleavage structure comprises detection of fluorescence.

31. (previously presented) The method of Claim 26, wherein said detecting the cleavage of said cleavage structure comprises detection of mass.

32. (previously presented) The method of Claim 26, wherein said detecting the cleavage of said cleavage structure comprises detection of fluorescence energy transfer.

33. (previously presented) The method of Claim 26, wherein said detecting the cleavage of said cleavage structure comprises detection selected from the group consisting of detection of radioactivity, luminescence, phosphorescence, fluorescence polarization, and charge.

34. (previously presented) The method of Claim 26, wherein said first nucleic acid molecule is attached to a solid support.

35. (previously presented) The method of Claim 26, wherein said second nucleic acid molecule is attached to a solid support.

36-41. (canceled)

42. (previously presented) The method of Claim 26, wherein said synthetic target nucleic acid comprises DNA.

43-60. (canceled)

61. (previously presented) The method of Claim 26, wherein said cleaving step is conducted under isothermal conditions.

62. (previously presented) The method of Claim 26, wherein said thermostable 5' nuclease comprises a 5' nuclease of a DNA polymerase.

63. (previously presented) The method of Claim 62, wherein said DNA polymerase is *Taq* DNA polymerase.

64. (previously presented) The method of Claim 26, wherein said 3' portion of said second nucleic acid molecule comprises an aromatic ring.

65. (previously presented) The method of Claim 26, wherein said 3' portion of said second nucleic acid molecule comprises a 3' terminal nucleotide not complementary to said target nucleic acid.

66. (previously presented) The method of Claim 26, wherein said 3' portion of said second nucleic acid molecule consists of a single nucleotide.

67. (previously presented) The method of Claim 66, wherein said single nucleotide is not complementary to said target nucleic acid.

68. (previously presented) The method of Claim 66, wherein said single nucleotide is complementary to said target nucleic acid.

69. (previously presented) The method of Claim 65, wherein said 3' terminal nucleotide

comprises a naturally occurring nucleotide.

70. (previously presented) The method of Claim 65, wherein said 3' terminal nucleotide comprises a nucleotide analog.

71. (previously presented) The method of Claim 26, wherein a plurality of said first nucleic acid molecule is provided, such that said first nucleic acid molecule is in concentration excess compared to said target nucleic acid.

72. (previously presented) The method of Claim 26, wherein a plurality of said second nucleic acid molecule is provided, such that said second nucleic acid molecule is in concentration excess compared to said target nucleic acid.

73. (previously presented) The method of Claim 26, wherein said target nucleic acid and said second nucleic acid molecule form a duplex, and wherein a plurality of said first nucleic acid molecule is provided such that said first nucleic acid molecule is in concentration excess compared to said duplex.

74. (previously presented) The method of Claim 73, wherein said cleaving said cleavage structure comprises cleaving said first nucleic acid molecule to generate non-target cleavage product.

75. (previously presented) The method of Claim 74, wherein said non-target cleavage product from said first nucleic acid molecule is generated in concentration excess compared to said duplex.

76. (previously presented) The method of Claim 26, further comprising providing a third nucleic acid molecule complementary to a third portion of said target nucleic acid upstream of said first portion of said first target nucleic acid, wherein said cleavage structure comprises said third nucleic acid molecule.

77. (previously presented) The method of Claim 26, wherein said thermostable 5' nuclease is a FEN-1 nuclease.

78. (previously presented) The method of Claim 26, wherein said 3' portion of said second nucleic acid molecule is completely complementary to said target nucleic acid.

79. (previously presented) The method of Claim 78, wherein during said incubating, said 3' portion of said second nucleic acid molecule partially displaces said portion of said first nucleic acid molecule that is completely complementary to said first region of said target nucleic acid.

80. (previously presented) A method for detecting the presence of a target nucleic acid molecule in a sample, comprising:

a) incubating a sample with a thermostable FEN-1 nuclease under conditions wherein a cleavage structure is formed, said cleavage structure comprising:

i) a synthetic target nucleic acid, said synthetic target nucleic acid comprising a first region and a second region, said second region downstream of and contiguous to said first region;

ii) a first nucleic acid molecule, wherein at least a portion of said first nucleic acid molecule is completely complementary to said first region of said target nucleic acid;

iii) a second nucleic acid molecule comprising a 3' portion and a 5' portion, wherein said 5' portion is completely complementary to said second region of said target nucleic acid;

wherein at least a portion of said first nucleic acid molecule is annealed to said first region of said synthetic target nucleic acid, and wherein at least a portion of said second nucleic acid molecule is annealed to said second region of said synthetic target nucleic acid;

b) cleaving said cleavage structure with said thermostable FEN-1 nuclease so as to generate non-target cleavage product; and

- c) detecting the cleavage of said cleavage structure.
81. (previously presented) The method of Claim 80, wherein said detecting the cleavage of said cleavage structure comprises detecting said non-target cleavage product.
82. (previously presented) The method of Claim 80, wherein said synthetic target nucleic acid comprises an amplified nucleic acid.
83. (previously presented) The method of Claim 82, wherein said amplified nucleic acid is produced using a polymerase chain reaction.
84. (previously presented) The method of Claim 80, wherein said detecting the cleavage of said cleavage structure comprises detection of fluorescence.
85. (previously presented) The method of Claim 80, wherein said detecting the cleavage of said cleavage structure comprises detection of mass.
86. (previously presented) The method of Claim 80, wherein said detecting the cleavage of said cleavage structure comprises detection of fluorescence energy transfer.
87. (previously presented) The method of Claim 80, wherein said detecting the cleavage of said cleavage structure comprises detection selected from the group consisting of detection of radioactivity, luminescence, phosphorescence, fluorescence polarization, and charge.
88. (previously presented) The method of Claim 80, wherein said first nucleic acid molecule is attached to a solid support.
89. (previously presented) The method of Claim 80, wherein said second nucleic acid molecule is attached to a solid support.

90. (previously presented) The method of Claim 80, wherein said synthetic target nucleic acid comprises DNA.

91. (previously presented) The method of Claim 80, wherein said cleaving step is conducted under isothermal conditions.

92. (previously presented) The method of Claim 80, wherein said 3' portion of said second nucleic acid molecule comprises an aromatic ring.

93. (previously presented) The method of Claim 80, wherein said 3' portion of said second nucleic acid molecule comprises a 3' terminal nucleotide not complementary to said target nucleic acid.

94. (previously presented) The method of Claim 80, wherein said 3' portion of said second nucleic acid molecule consists of a single nucleotide.

95. (previously presented) The method of Claim 94, wherein said single nucleotide is not complementary to said target nucleic acid.

96. (previously presented) The method of Claim 94, wherein said single nucleotide is complementary to said target nucleic acid.

97. (previously presented) The method of Claim 93, wherein said 3' terminal nucleotide comprises a naturally occurring nucleotide.

98. (previously presented) The method of Claim 93, wherein said 3' terminal nucleotide comprises a nucleotide analog.

99. (previously presented) The method of Claim 80, wherein said 3' portion of said second nucleic acid molecule is completely complementary to said target nucleic acid.

100. (previously presented) The method of Claim 99, wherein during said incubating, said 3' portion of said second nucleic acid molecule partially displaces said portion of said first nucleic acid molecule that is completely complementary to said first region of said target nucleic acid.

101. (previously presented) The method of Claim 80, wherein a plurality of said first nucleic acid molecule is provided, such that said first nucleic acid molecule is in concentration excess compared to said target nucleic acid.

102. (previously presented) The method of Claim 80, wherein a plurality of said second nucleic acid molecule is provided, such that said second nucleic acid molecule is in concentration excess compared to said target nucleic acid.

103. (previously presented) The method of Claim 80, wherein said target nucleic acid and said second nucleic acid molecule form a duplex, and wherein a plurality of said first nucleic acid molecule is provided such that said first nucleic acid molecule is in concentration excess compared to said duplex.

104. (previously presented) The method of Claim 103, wherein said cleaving said cleavage structure comprises cleaving said first nucleic acid molecule to generate non-target cleavage product.

105. (previously presented) The method of Claim 104, wherein said non-target cleavage product from said first nucleic acid molecule is generated in concentration excess compared to said duplex.

106. (previously presented) The method of Claim 80, further comprising providing a third nucleic acid molecule complementary to a third portion of said target nucleic acid upstream of said first portion of said first target nucleic acid, wherein said cleavage structure comprises said third nucleic acid molecule.

107. (canceled)